THE SIZE AND SHAPE OF METAMORPHOSING LARVAE OF VENUS (MERCENARIA) MERCENARIA GROWN AT DIFFERENT TEMPERATURES

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Among the characters to be employed for identification of lamellibranch larvae, their size at metamorphosis has been suggested by some workers (Lebour, 1938). Other students have maintained, however, that since there may be correlations between size and environmental factors, the size at metamorphosis cannot be used as a criterion for recognition of larvae. Sullivan (1948) thought that this was confirmed for mature larvae of *Mya arenaria* in Canadian waters, which she observed to metamorphose in Malpeque Bay upon reaching a length of 250 microns, as compared with a length of 415 microns reported by Stafford (1912) for larvae of the same clam in the St. Andrews region.

According to Sullivan the difference in size at setting was probably the result of the difference in the summer water temperature at these two places because larvae developing at the lower temperature (St. Andrews) needed to reach a larger size before metamorphosing. This opinion is, indirectly, in agreement with that of Erdmann (1934), who thought that the size of larvae of the European oyster, *Ostrea edulis,* at the time of swarming was regulated by the temperature at which the larvae developed.

So far, no one has offered a definite explanation as to why lamellibranch larvae should reach a larger size if grown in colder water. It seems, however, that this opinion was formed because it is widely believed that, "In many instances, and perhaps as a general rule, the size that an animal attains is greater when it is reared at low temperature" (Coker, 1947, p. 102). Furthermore, since it is known that large individuals of the same species have a relatively smaller surface area than do small ones, the larger size may be considered as an adaptation to an increase in the viscosity of the water which accompanies a decrease in temperature. Examples to support this assumption can be found in numerous papers, including those of Murray and Hjort (1912), and many students of Radiolaria, copepods and certain other forms (Hedgpeth, 1957). Kofoid (1930), for instance, reported that marine protozoa living in cold water grow to much larger sizes than do their relatives existing at higher temperatures.

Aquatic biology also offers many instances in which organisms of the same species grown at different temperatures may show a somewhat different shape. The phenomenon of cyclomorphosis, reviewed by Brooks (1946), is an example. In lamellibranch larvae, of course, no radical changes in structure, as observed in Daphnia populations, can be anticipated. Yet, it has been reported (Jørgensen, 1946, p. 296) that at least in some lamellibranchs, such as *Venus gallina,* "the larval outline varies from almost square to circular." According to the same author

the veligers of the common mussel, *Mytilus edulis,* of Danish waters also show a remarkable variability in their shape. The water temperature during development can again be suspected as a factor affecting the shape of the larva.

During the past few years larvae of approximately 20 species of lamellibranchs have been successfully cultured from fertilized egg through metamorphosis by members of our laboratory (Loosanoff, 1954). The data collected during these studies will soon permit us to offer reliable material for recognizing larvae of the species with which we have been working. It will include photomicrographs of the larvae and length-width measurements of their shells from early straight hinge stage until metamorphosis. However, before offering these criteria, it was deemed necessary to ascertain the following possibilities, which could reflect on the reliability of our material:

1) We wanted to know whether, as is suggested by some students, individuals of larval populations grown at relatively low temperatures reach a larger size before metamorphosing than do larvae grown in warmer water. If this is true, special corrections, perhaps as formulae, should be offered to show the relationship between average size at setting and water temperature.

2) Since one of the criteria for recognizing a larva nearing metamorphosis is its dimensions, *i.e.,* length and width, it was necessary to determine whether the length-width ratio is relatively constant or if it changes in conformance with the temperature of the water in which larvae develop.

The questions posed above could be answered only on the basis of well-controlled experiments in which the water temperature was the only factor varied. Obviously, because of the time and efforts required, it would have been difficult to conduct such experiments with larvae of all 20 species of lamellibranchs with which we were working. We decided, therefore, to limit ourselves to observations on one or two species only. This paper is devoted chiefly to a description of the observations on size and length-width ratio at the beginning of metamorphosis of larvae of the hard shell clam, *Venus* (*Mercenaria*) *mercenaria,* developing at different temperatures.

Certain aspects of the studies which provided data for this paper have already been described (Loosanoff, Miller and Smith, 1951). In brief, they consisted of growing larvae of *Venus* (*Mercenaria*) *mercenaria* at constant temperatures ranging from 15.0° to 33.0° C. at intervals of 3.0° C. Since fertilized eggs that were placed in water of 15.0° or 33.0° C. showed abnormal development and heavy mortality, few ever reaching veliger stage, growth of larvae at these temperatures will not be discussed here.

The work was done in winter, the time we find most convenient to control the water temperature (Loosanoff, 1949). Altogether, four experiments were conducted. However, in one experiment one of a pair of cultures grown at 24.0° C. was accidentally lost, while in the fourth experiment, which was conducted during a comparatively warm spell when low temperature was difficult to maintain, no cultures were carried at 18.° C. As is our practice, the water in the culture jars was changed every second day (Loosanoff and Davis, 1950). The larvae were fed a mixture of micro-organisms consisting principally of *Chlorella* sp.

Samples for larval measurements were taken 48 hours after fertilization and every second day thereafter, until metamorphosis. These samples consisted of 50 larvae measured at random from each culture vessel, *i.e.,* 100 larvae from each temperature group. The length represented the greatest distance between the anterior and

posterior shell margins, while the width was the distance measured from the tip of the umbo to the middle of the ventral shell margin.

Because the larvae could not be marked individually, their rate of growth and size at setting could not be recorded directly on this basis. For this reason we used two substitute criteria. One was the average length of the larvae on the day the setting was first observed in each culture, and the second, the maximum size of the larvae observed during the life of the culture.

The number of days required for setting to begin at the different temperatures in the four experiments is given in Figure 1. Clearly enough, there were differences between the cultures within the same temperature group and also between

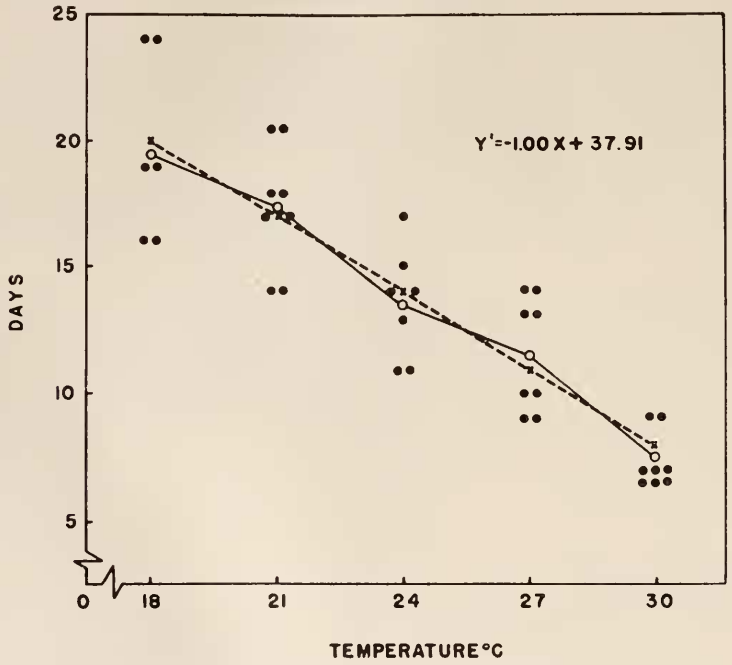

FIGURE 1. Number of days necessary for clam larvae to begin setting in cultures grown at different temperatures. ● = mean of individual culture; o = mean of all cultures grown at the same temperature; x = mean for given temperature predicted from regression line.

the groups carried at different temperatures. The most uniform results were obtained with the 30.0° C. group, where the beginning of setting in the different cultures varied between seven and nine days after fertilization, a difference of only two days. However, the difference between the shortest and longest periods needed for larvae to begin setting became greater in colder water. For example, in the 18.0° C. group the earliest beginning of setting was recorded 16 days after fertilization and the latest, after 24 days, a difference of eight days (Fig. 1).

An analysis of variance was carried out to test the significance of the differences among the different temperature groups on the number of days required for setting to begin. Since the result was highly significant (beyond the .001 level), separate

"t" tests were run for all possible pairs of temperature groups. All the "t" tests were highly significant (beyond the .01 level), with exception of the comparison between the 27.0° and 24.0° C. groups, and between the 21.0° and 18.0° C. groups. These results show, therefore, that a very strong relationship exists between temperature and date of setting, *i.e.*, larvae reared at high temperatures set significantly

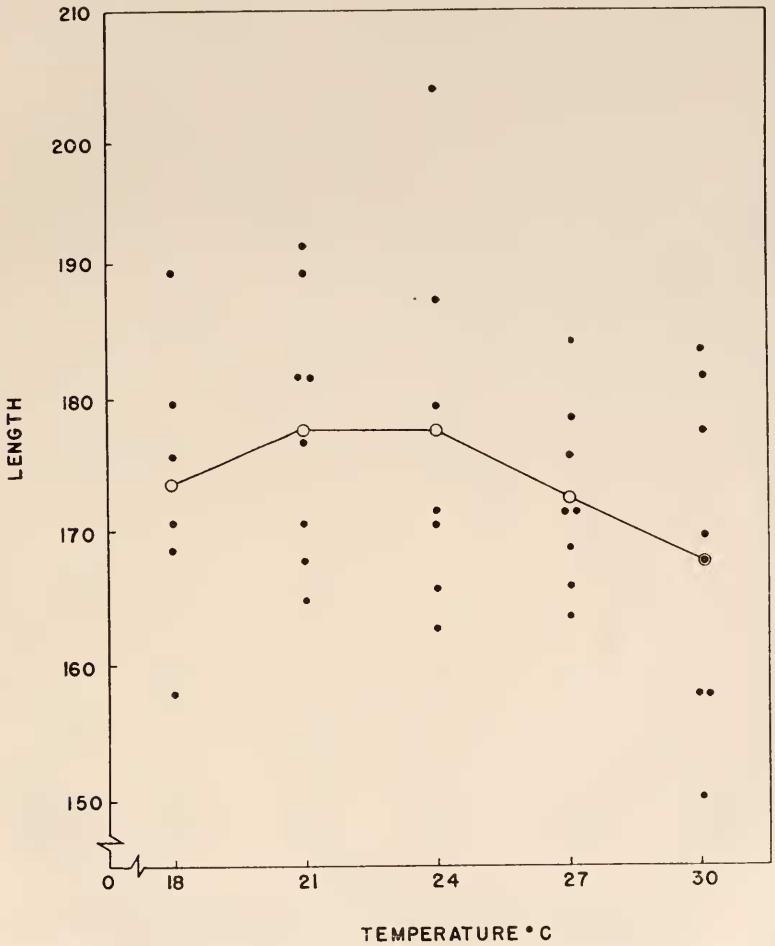

FIGURE 2. Mean length of clam larvae grown at different temperatures on day of beginning of setting. Measurements in microns. ● = mean of individual culture; o = mean of all cultures grown at the same temperature.

earlier than those raised at lower temperatures. This conclusion was expressed in the preliminary paper (Loosanoff, Miller and Smith, 1951).

Plotting of the dates of beginning of setting in the different cultures against the temperatures showed that the mean number of days for setting to begin for the various temperatures lies on an almost straight line (Fig. 1). Since the line con-

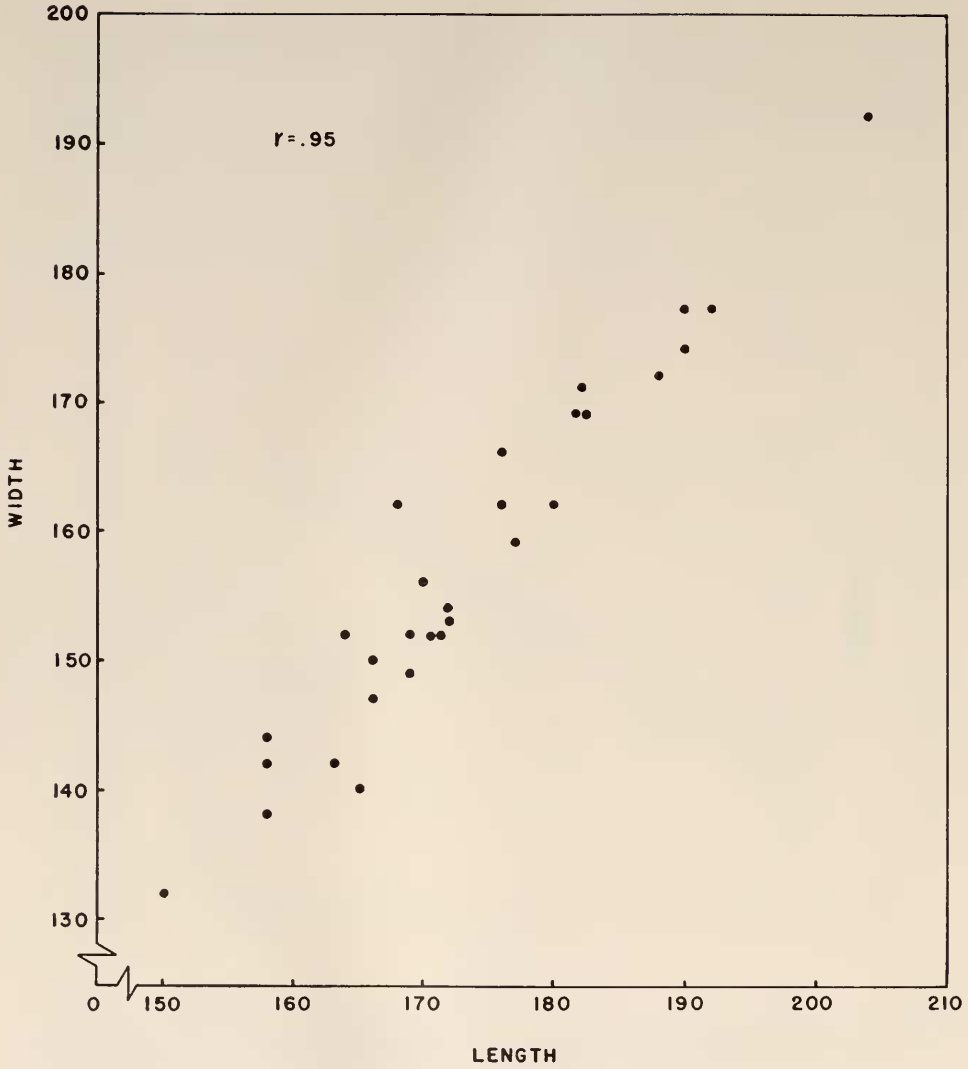

FIGURE 3. Mean length and width of clam larvae of individual cultures on day of beginning of setting. Measurements in microns.

necting the means of the different temperatures is obviously rectilinear, a regression equation was computed and found to be $Y' = -1.00\,X + 37.91$, where Y' is the predicted setting date and X is the temperature.

Because the regression line is an excellent fit to the experimental data, it is probable that interpolation within the 18.0° to 30.0° C. limits of the experiment can be made with a fair degree of confidence. However, extrapolation beyond these limits is not justifiable. This was shown by our other experiments, which demonstrated that clam eggs placed in water having a temperature of 15.0° or 33.0° C.

did not develop normally. Therefore, since the lineal regression does not hold even for a slightly higher or lower temperature, it cannot be expected to hold for lower or higher temperatures.

An analysis of variance test showed no significant differences among the five temperature groups, with respect to mean length of larvae at date of setting. Thus, although larvae grown at different temperatures required different periods to reach metamorphosis, in all cases they reach approximately the same mean length before setting. This observation indicates, therefore, that there was virtually no relationship between temperature and mean length at date of setting (Fig. 2). Nevertheless, the same figure shows that there was considerable variation in mean length at the beginning of setting among the various cultures within each temperature group.

In our studies we were also concerned with the shape, at metamorphosis, of larvae grown at different temperatures because, as has already been mentioned, the literature contains several remarks concerning variability of shape of larvae of the same species near setting time. Since the simplest method of describing the shape of a larva in mathematical terms for statistical analysis is to indicate its length-width ratio, measurements were made on larvae of all cultures, except those constituting the fourth experiment where no width measurements were taken, and the correlation between the mean length and the mean width of the larvae of each culture, on the day of the beginning of setting, was determined (Fig. 3). The

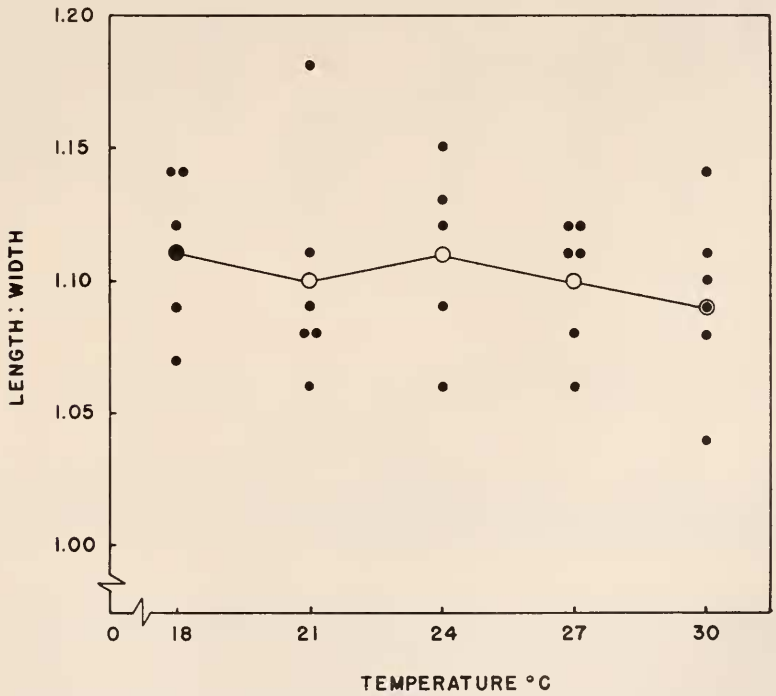

FIGURE 4. Ratio of mean length to mean width of clam larvae of different cultures on days of beginning of setting, in relation to temperature. ● = mean of individual culture; o = mean of all cultures grown at the same temperature.

results indicated this correlation to be so high ($r = .95$) that it seems unlikely that any analysis made using width as a variable would add anything new to that already made with length.

Continuing the analysis of data that might help in discovering the differences in shape of larvae grown at different temperatures, a study was made of the ratio of mean length to mean width at the date of setting. It failed to show the existence of any significant change in the ratio at different temperatures (Fig. 4). Thus, this matter has been satisfactorily solved to assure investigators working with lamelli-

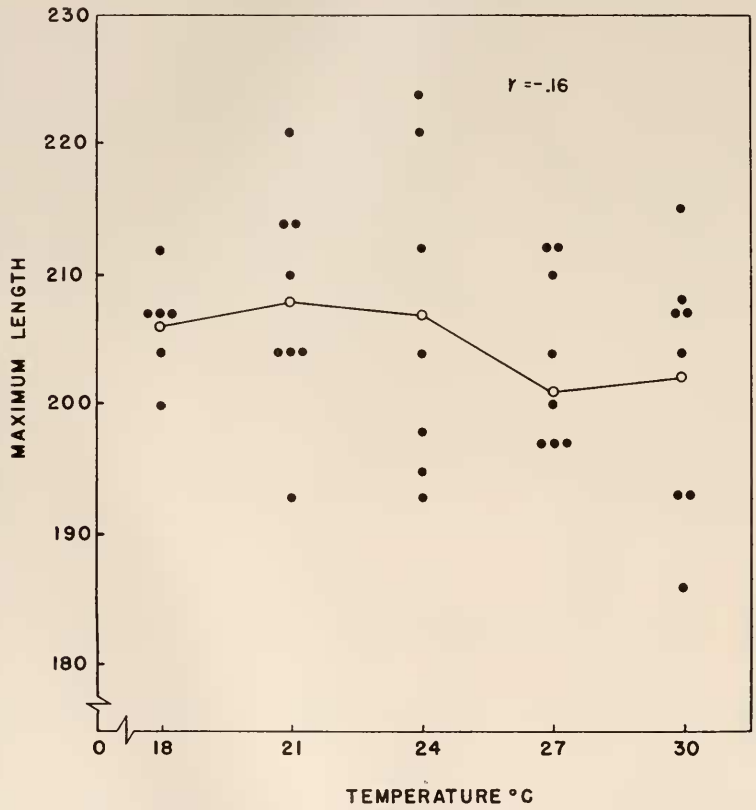

FIGURE 5. Maximum length of clam larvae of different cultures during the life of a culture, in relation to temperature. Measurements in microns. ● = mean of individual culture; o = mean for all cultures grown at the same temperature.

branch larvae that individuals of the same species display virtually the same shape at metamorphosis, even though they are grown at different temperatures.

Although our studies showed that even if larvae are grown at different temperatures, they, in all cases, reach approximately the same mean length before setting, the question still unanswered is whether there is an appreciable relationship between the *maximum* length of larvae on the date of setting and the temperature. A statistical analysis demonstrated the lack of an appreciable relationship

between these two variables, giving a correlation of $-.16$. The lack of relationship is clearly indicated in Figure 5, which shows that the means of the cultures grown at five different temperatures varied from 201 μ to 208 μ, a range of only seven microns. Nevertheless, we again noticed a considerable variability among the cultures within each temperature group, although it was much less pronounced within the 18.0° C. group than in certain others. However, again, no definite trend in this respect was observed because the variability of the maximum length of the

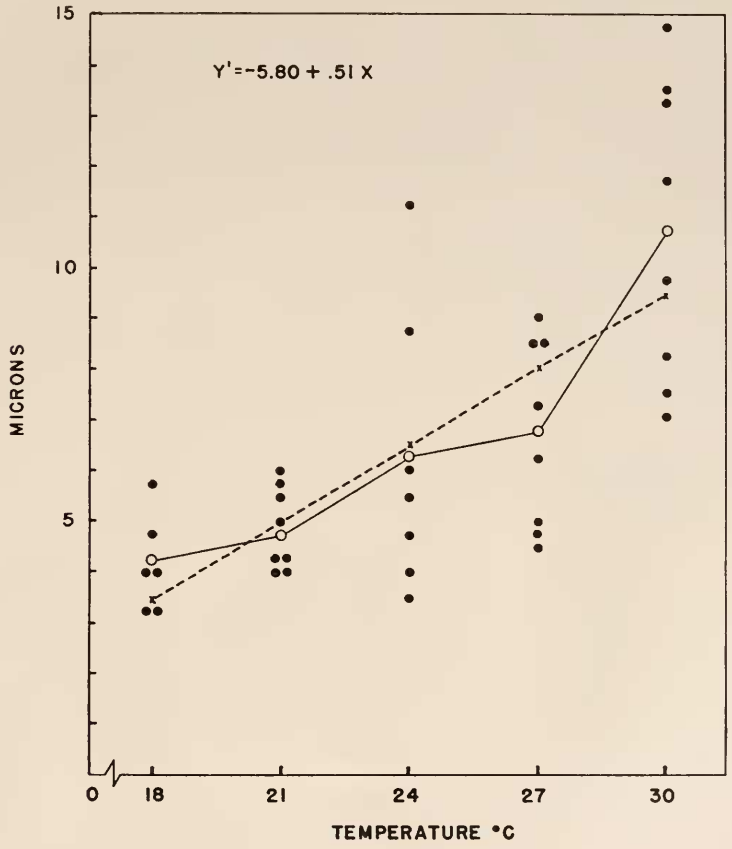

FIGURE 6. Average daily length increment of larvae grown at different temperatures. Measurements in microns. ● = mean of individual culture; o = mean of all cultures grown at the same temperature; x = mean for given temperature predicted from regression line.

larvae in the different cultures grown at 27.0° C., the second highest temperature group, did not differ greatly from that recorded for the lowest, *i.e.*, the 18.0° C. group.

Using the data collected during these studies, we can calculate the average daily growth increment for all cultures at given temperatures. By plotting the average daily growth increment for each temperature group against the corresponding

temperature, an approximately rectilinear relationship becomes evident (Fig. 6). The regression equation computed was found to be $Y' = -5.80 + .51\ X$, where Y' is the predicted average daily growth increment and X is the temperature. The relationship indicates, naturally, that faster growth occurred at higher temperatures and, theoretically, from the regression equation it can be assumed that if the temperature were reduced to about 11.3° C., growth would stop completely.

In discussing our results it must be remembered that they are representative only for this series of experiments and that there are many factors which can change the daily growth increment. One of these, capable of lowering or increasing the rate of growth of larvae, is the quality of the food. In our experiments the larvae were fed a mixture of phytoplankton, consisting largely of *Chlorella.* However, recent studies of Davis and Guillard (1958) clearly showed that the growth of clam larvae varied greatly depending upon the kind of food they were given. Davis found that the larvae grew best when fed a culture of mixed flagellates, including *Isochrysis, Monochrysis, Dunaliella,* and *Platymonas,* while the larvae given *Chlorella,* two species of which were tried, grew considerably slower. In the same series of experiments Davis was able to demonstrate that the rate of growth of larvae of the American oyster, *Crassostrea virginica,* also varied greatly according to the kind of food organisms available.

Quantity of food organisms is another factor to be considered. Our earlier work (Loosanoff and Engle, 1947; Loosanoff, Davis and Chanley, 1953a) showed that heavy concentrations of food cells, such as *Chlorella,* usually seriously interfered with the feeding of adult oysters and that they also either killed the clam larvae or retarded their growth. Furthermore, they indicated that the optimum concentration of food organisms depended upon the kind and size of their cells. Since, in our experiments described in this article, the number of cells was not accurately determined and the food did not consist of pure cultures but of a mixture of many organisms, we do not know whether the clam larvae were fed the optimum food concentrations. This circumstance, however, does not invalidate our comparisons because all cultures were given food of the same quality and in the same quantity.

Finally, the effect of the concentration of larvae in the experimental cultures should be considered. Our studies (Loosanoff, Davis and Chanley, 1953b; Loosanoff, 1954) have shown that larvae in crowded cultures grow somewhat slower. However, the difference in the rate of growth of larvae in lightly-populated and those in densely overcrowded cultures was not too great. For example, we determined, at the end of the tenth day, that the mean length of larvae in the cultures containing only six individuals per cubic centimeter of water was 162 μ, whereas the mean length in the overcrowded cultures containing 52 individuals per cubic centimeter was 144 μ, or only 18 μ less than that recorded for lightly-populated cultures. Since, in the experiments described here, we began with the same number of larvae in all containers and because during the experiments no excessive mortality was recorded in any of the cultures, our larval populations in all jars were not much different from each other and, therefore, could not seriously affect the uniformity of the experimental conditions.

In concluding this article a brief reference to one more aspect of the role of water temperature on growth of bivalve larvae may be appropriate. It has frequently been reported that species living in warmer water have just as long a pelagic life as their

northern relatives, and that, at a given temperature, the eggs of the southern species cleave and develop more slowly than those of the northern species of the same genus (Fox, 1936; Thorson, 1950). This suggests that even if the eggs and larvae were cultured under identical conditions, development of the eggs and larvae of the southern clam, *Venus* (*Mercenaria*) *campechiensis,* would require a longer period than is needed for eggs and larvae of the northern clam, *Venus* (*Mercenaria*) *mercenaria.* I had the opportunity to verify this contention by the studies conducted together with my associate, H. C. Davis. Adult *Venus* (*Mercenaria*) *campechiensis* were imported from the Apalachicola area of the Gulf of Mexico in November, 1953. Several weeks later these clams were conditioned for spawning. A group of large *Venus* (*Mercenaria*) *mercenaria,* natives of Long Island Sound, were ripened under identical conditions simultaneously with the southern species. When both groups were ripe, spawning was induced by our usual methods (Loosanoff and Davis, 1950). Fertilized eggs of each species and, later, larvae developing from these eggs were cultured under identical conditions, the temperature being approximately 21.0° C. Triplicate cultures of each species were grown, and random samples of 100 larvae from each culture were measured every second day. The curves constructed on the basis of this information showed that the rates of growth of the larvae of the two species were practically identical. Moreover, setting of larvae of both groups began at the same time. The results of this experiment contradict, therefore, the conclusion that when grown at the same temperature the eggs and larvae of the southern species develop more slowly than those of the northern species of the same genus.

I wish to express my thanks to Mrs. Barbara Myers for the statistical analysis of the data and to my associates, Miss Rita S. Riccio and Harry C. Davis, for their help in preparation of this article.

Summary

1. The mean setting dates for larvae of *Venus* (*Mercenaria*) *mercenaria* grown at constant temperatures of 30.0°, 27.0°, 24.0°, 21.0° and 18.0° C. were found to lie on an almost perfectly straight line according to equation $Y' = -1.00X + 37.91$, where Y' is the predicted setting date and X is the temperature.

2. There were no significant differences among the five temperature groups with respect to mean length of larvae at time of setting.

3. There was no apparent relationship between *maximum* length of larvae at time of setting and temperature.

4. The correlation between mean width and mean length of larvae at time of setting was very high ($r = .95$).

5. No apparent relationship was found between shape of larvae (*i.e.*, ratio of mean length to mean width) at time of setting and temperature.

6. The average daily growth increment for all cultures at given temperatures under the conditions prevailing during the experiments was determined.

7. The rate of growth of larvae of the southern clam, *Venus* (*Mercenaria*) *campechiensis,* was the same as that of the northern species, *Venus* (*Mercenaria*) *mercenaria,* when the temperature and other conditions were identical. Moreover, setting of larvae of both species began at the same time.

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